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A survey of assimilable organic carbon, biodegradable organic carbon and coliform growth response in US drinking waters

Résumé sur le carbone organique assimilable (COA), le carbone organique biodégradable (COB) et l'indice de croissance des coliformes (ICC) dans l'eau potable aux Etats-Unis.

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Résumé de l'article

Les objectifs principaux de cette étude étaient :

1) De documenter, par une étude de large envergure, les concentrations en COA de l'eau potable, et des usines de traitement.

2) De comparer l'indice de croissance des coliformes (ICC) aux concentrations en COA.

3) De comparer les concentrations en CODE et celles de COA.

Le COA de l'eau a été mesuré avec un mélange de cultures *Pseudomonas fluorescens* de souche P17 et de *Spirillumsp.* de souche NOX. La plupart des échantillons d'eaux ont été transferrés dans des ampoules de 40 ml, stérilisés puis inoculés avec les bactéries *Pseudomonas* et *Spirillum* et incubés à 15 °C. Les unités formant colonies représentaient le paramètre de suivi.

Le test relatif à l'ICC a été réalisé avec l'eau soumise au test, préalablement stérilisée puis inoculée avec *Cloacae enterobacter*. Les échantillons ont été incubés à 20 °C pendant 5 jours et la croissance des coliformes mesurée par les unités formant colonies.

La mesure de CODB a été exécutée dans des ampoules de 40 ml : l'échantillon à analyser, préalablement filtré sur fibre de verre et pasteurisé, était inoculé avec une microflore indigène de rivière puis incubé pendant 28 jours à l'obscurité à température ambiante.

L'étude a porté sur 109 échantillons prélevés en 79 points d'approvisionnement en eau potable répartis sur le territoire des Etats-Unis et du Canada : 26 d'eaux souterraines, et 53 d'eaux de surface.

Des flacons de prélèvements pour échantillonner et des instructions pour prélever et pasteuriser les eaux ont été fournis à tous les techniciens.

L'eau stérilisée était envoyée à la Stroud Water Research Centre pour les analyses du COA, CODB et COD et au Risk Reduction Laboratory pour l'essai relatif à l'indice de croissance des coliformes. Les densités des bactéries coliformes et des bactéries hétérotrophes sur gélose étaient mesurés par les techniciens eux-mêmes.

Les concentrations en COD s'échelonnaient de 203 à 4943 µg/L, celles en COA de 18 à 322 µg/L (représentant ainsi de 2,4 % à 44,0 % du COD).

Les valeurs élevées du pH dans 5 échantillons d'eau ont inhibé la croissance des bactéries soumises aux deux essais biologiques (AOC ou BDOC).

Les concentrations en CODB se sont échelonnées de 1 à 1521 µg/L (soit 0,4 % à 52,8% du COD).

L'essai sur l'indice de croissance des coliformes a montré que 79 % des eaux soumises au test n'ont pas permis la croissance des coliformes alors que 7 % entraînaient une forte croissante (toutes provenant d'eaux de surface) et 14 une croissance modérée.

Les services des eaux n'ont mis en évidence aucun coliforme et seulement une faible densité de bactéries hétérotrophes dans les eaux des usines de traitement.

La corrélation COA-CODB était significative ($P \ll 0{,}01$) avec un coefficient de corrélation de $r = 0{,}594$.

Des corrélations significatives COA-COD et CODB-COD ont également été mises en évidence.

Par contre, les corrélations ICC-COA ou ICC-CODB n'étaient pas significatives.

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Résumé sur le carbone organique assimilable (COA), le carbone organique biodégradable (COB) et l'indice de croissance des coliformes (ICC) dans l'eau potable aux Etats-Unis

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Reçu le 12 novembre 1991, accepté pour publication le 24 août 1992*.

SUMMARY

The primary objectives of this study were : 1) to document concentrations of Assimilable Organic Carbon (AOC) in a survey of a broad range of drinking waters and treatment processes ; 2) compare the Coliform Growth Response (CGR) to AOC concentrations ; and 3) compare Biodegradable Dissolved Organic Carbon (BDOC) concentrations to AOC concentrations. AOC was measured with mixed cultures of *Pseudomonas fluorescens* strain P-17 and *Spirillum sp.* strain NOX. Test waters were transferred to 40-ml vials, pasteurized, inoculated, and incubated at 15°C. Colony forming units was the test parameter. CGR was performed with pasteurized test water inoculated with *Enterobacter cloacae*. Samples were incubated at 20°C for 5 days and response determined from colony forming units. The BDOC assay was performed in 40-ml vials, with glass fiber filtered, pasteurized test water, inoculated with the indigenous microflora from a stream, and incubated for 28 days in the dark at room temperature. The survey involved 109 samples from 79 drinking water supplies located throughout the United States and Canada, including 26 groundwater and 53 surface water sources. Utility personnel were supplied with sample bottles and instructions for sampling and pasteurizing the test waters. Pasteurized water was sent to the Stroud Water Research Center for AOC, BDOC, and DOC analyses, and to the Risk Reduction Engineering Laboratory for the CGR assay. Densities of coliforms and heterotrophic plate count bacteria (HPC) were measured in the test waters by utility personnel. DOC concentrations ranged from 203 to 4943 µg/L. AOC concentrations ranged from 18 to 322 µg/L, or 2.4 % to 44.0 % of the DOC. High pH values in

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5 test waters inhibited the growth of both AOC bioassay organisms. BDOC concentrations ranged from 1 to 1521 µg/L, or 0.4 % to 52.8 % of DOC. The CGR assay indicated that 79 % of the test waters did not promote coliform growth, 7 % were strongly growth promoting, all from surface water sources, and 14 % were moderately growth promoting. No coliforms and only low densities of HPC organisms were reported by utilities for treatment plant effluents. The correlation of AOC and BDOC was significant ($P<0.01$), with a correlation coefficient of $r=0.594$. Significant correlations were also found for AOC and DOC, and BDOC and DOC. Correlations of CGR and either AOC or BDOC were not statistically significant.

Key-words • *AOC, BDOC, DOC and CGR.*

RÉSUMÉ

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Mots clés : *COA, CODB, COD, ICC, croissance bactérienne.*

INTRODUCTION

The growth of bacteria in drinking water distribution and storage systems can lead to the deterioration of water quality (O'CONNER *et al.*, 1975), including taste and odor (RIZET *et al., 19*82), elevated turbidity (Olson, 1982), and non-compliance with the U. S. EPA Coliform Rule. Additionally, growth or regrowth can reduce hydraulic capacity, accelerate corrosion (LEE *et al.*, 1980), and make it more difficult to maintain disinfectant residuals (CHARACKLIS, 1988). Regrowth of bacteria is a multifaceted phenomenon influenced by biodegradable organic matter (VAN DER KOOIJ *et al.*, 1982), inorganic nutrients, efficacy of residual disinfectants (LECHEVALLIER *et al.*, 1988), temperature regime (FRANSOLET *et al.*, 1985), residence time in mains and storage units (MAUL *et al*, 1985), pH, redox potential, inoculum size, shear stress, and main construction material (COLBOURNE *et al.*, 1988).

Concentrations of biodegradable organic matter are important to the regrowth problem in distribution systems because bacteria of public health significance are heterotrophs. Heterotrophs derive energy and carbon for growth and reproduction from biodegradable organic matter, a subset of the dissolved organic carbon (DOC) molecules in the source water (GOTTSCHALK, 1986). Comprehensive studies with data from pilot scale experiments and full-scale distribution system data will be required to improve the ability to predict when conditions will lead to regrowth problems. Effluent criteria for treated water based upon model predictions will help the industry to control microbial growth in distribution systems. There can be little doubt that when effluent criteria are proposed, the concentration of biodegradable organic matter will be a criterion.

Methods for the measurement of biodegradable organic matter and bacterial regrowth potential in drinking water have recently been reviewed (HUCK, 1990). Our work focuses on three different methods which we will briefly describe. A bioassay wherein the growth of a test organism(s) is used to

quantify the concentration of biodegradable organic matter present in drinking water has been developed and referred to as assimilable organic carbon (AOC) by VAN DER KOOIJ *et al.* (1982). A bioassay developed by HASCOET *et al.* (1986), and modified by JORET and LEVI (1986) is based on the activity of the indigenous microflora, particularly the ability to catabolize organic carbon to CO_2. This DOC based assay is referred to as the biodegradable dissolved organic carbon (BDOC) assay. A third method, similar in principle to the AOC bioassay, but using a coliform as the bioassay organism has been developed by RICE *et al.* (1990) and is called the Coliform Growth Response (CGR).

The AOC technique has the advantages of using a standard inoculum and being reproducible. It also is advantageous to have an assay with a response parameter (cell density) which exceeds the initial conditions (inoculum density) by several orders of magnitude. The AOC technique is extremely sensitive, 1 µg/L can produce a "signal" of 1 x 10^4 cfu/mL, yet blank values below 5 to 10 µg/L are readily achieved. The AOC method has the disadvantages of relying upon the metabolic capabilities restricted to two species, occasional inhibition of the test organisms by the test water, and providing AOC concentrations in units of acetate or oxalate-carbon equivalents.

The BDOC assay combines microbiological and chemical techniques, measuring DOC concentrations before and after exposure of the test water to a bacterial inoculum to estimate biodegradable organic matter concentrations. As an alternative to the AOC measurement, the BDOC assay has the advantage of relying upon the catabolic activities of numerous bacteria within a heterogenous inoculum rather than the growth of 2 species. The BDOC assay response parameter is DOC concentration, and another advantage is that concentrations of non-labile or refractory DOC are also estimated. A disadvantage of the BDOC assay is sensitivity, because the change in DOC concentration during incubation can be small relative to a large background.

The CGR assay, using *Enterobacter cloacae* as the test organism, is particularly relevant to drinking waters in North America because it targets the group of organisms specified in US EPA drinking water regulations. Cell numbers is the test parameter in the CGR assay, and the assay provides an index of the ability of the test water to support coliform growth. The advantage of the CGR assay is the focus on coliforms. The disadvantage of a focus limited to coliforms is that it does not permit a more general prediction of regrowth for other heterotrophic bacteria. Heterotrophic regrowth probably occurs at a lower nutrient threshold than colifom regrowth and could produce conditions conducive to the growth of coliforms.

To date there has not been a survey of AOC concentrations from a wide variety of drinking waters in North America, or a rigorous comparison of the AOC, BDOC, and CGR procedures on numerous test waters. The primary objectives of this study were to : 1) document concentrations of AOC in a survey of drinking waters and treatment processes ; 2) compare the CGR to AOC concentrations ; 3) compare BDOC concentrations to AOC concentrations ; and 4) determine if any of the above water quality parameters were related to concentrations of coliforms or heterotrophic plate count (HPC) organisms in treatment plant effluent waters.

METHODS

Selection of participating utilities

Questionnaires and letters of solicitation were mailed to 177 water utilities across the United States and Canada, asking for information on source waters, treatment process, and the availability of supplemental data, especially coliform and HPC densities. The responses were tabulated and only those utilities that performed HPC analyses in addition to coliforms were selected.

Glassware preparation

All glassware was borosilicate and we rendered it organic carbon free by combustion at 550 °C for 6 hours. We used 1-L and 500-mL collection bottles (Schott) with teflon lined screw caps, and 40-mL incubation vials with teflon backed silicone septa. The teflon liners and septa were cleaned by soaking in 10 % sodium persulfate at 60°C for 0.5 hours.

Water sampling by participating utilities

Approximately 2 weeks prior to the scheduled sampling date, utilities were sent all the materials necessary for their participation in the survey. The materials included an instruction sheet, collection bottles containing sodium thiosulfate, bottle labels, sample voucher sheets, sample data sheets, mailing labels, and mailing boxes. The utilities were instructed to fill the sample bottles at the time that they were performing routine monitoring for coliforms and HPC. The bottles were filled to the shoulder without rinsing, closed tightly, and shaken to mix the thiosulfate. Utilities placed the bottles into a water bath, heated them to 70 °C, and maintained that temperature for 0.5 h. After heat fixing, the bottles were removed from the water bath, placed into an ice bath, and cooled to room temperature. The bottles were then dried, the labels affixed, and the bottles were placed into the mailing boxes with the sample voucher and sent to either the U.S. EPA or the Stroud Water Research Center by overnight express mail. The EPA received one 500-mL bottle, and the Stroud Center received two 1-L bottles. When the data became available, the utilities sent us the results of their coliform and HPC data for the sampling date.

Analysis of the test waters

At the Stroud Center we analyzed the samples for DOC, AOC, and BDOC, and at the Risk Reduction Engineering Laboratory we performed the CGR assay. At the Stroud Center we poured 3 subsamples from the two 1-liter bottles into 40-mL vials to be analyzed for DOC measurements using platinum catalyzed persulfate oxidation (O.I. 700). The precision of DOC analyses based upon 2 mg/L standards is between 7 and 10 µg C/L (KAPLAN, 1992). An additional 25 subsamples of the test water were poured into vials that were closed tightly with screw caps, teflon backed silicone septa (Pierce), and septa guards (I-Chem). We placed the 25 vials and the collection bottles with their remaining water into a 70 °C waterbath for a second heat fixation. While the

water in the collection bottles was still warm, we filtered it into 10 vials for the BDOC assay. We filtered the water using a 60-mL polypropylene syringe and a double thickness of carbon free glass fiber filters (Whatman GFF) in an acetal resin syringe type filter holder and closed the vials with septa and screw caps.

The AOC assay was the method of VAN DER KOOIJ *et al.* (1982) as modified by KAPLAN and BOTT (1989). *Pseudomonas fluorescens* strain P-17 and *Spirillum* strain NOX were grown in separate 250-mL Erlenmeyer flasks with ground glass stoppers containing sterile stream water amended with 1 mg acetate-C/L. Densities of the cells were enumerated by epifluorescent direct microscopic counts and diluted with the test water so that a 50 µl inoculum would result in 500 cfu/mL of each species in 40 mL. Inoculation was performed through the septa using a Tuberculin syringe and stepper pipette (Tridak). The vials were stored in the dark at 15 °C, and after 7, 8, and 9 days, 8, 8, and 9 vials per sample day, respectively, were sampled and serially diluted for spread plates on R2A nutrient agar.

The BDOC assay was similar to the method of HASCOET *et al.* (1986). For an inoculum, we filtered stream water through a single glass fiber filter (Whatman GFF) and sonicated the filtrate at 50 W for 30 sec (Branson) to kill all protozoans. A subsample of the inoculum was placed into bacterized Cerophyl to verify the absence of protozoa. A 400 µl inoculum was injected through the septa and the vials were incubated in the dark at room temperature for 28 days. Five vials which were not inoculated were analyzed for the initial DOC concentration, and after 28 days the water in 5 inoculated vials was filtered through a double thickness of glass fiber filters and analyzed for DOC.

The CGR assay was the method of RICE *et al.* (1991), except that the pasteurized water received from the utilities was used without further treatment. *Enterobacter cloacae* was acclimated to low nutrient conditions after growth in 1 % heart infusion for 18 h at 35 °C. The culture was washed twice and then resuspended in phosphate buffer at 20 °C for 24 h. Acclimated cells were inoculated into 2 different sized incubation vessels, 40-mL vials with teflon-backed septa and 250-mL glass bottles with ground-glass closures. The incubation vessels were incubated at 20 °C for 5 days and the number of organisms present was enumerated by the spread plate procedure on plate count agar. The CGR was determined from a $\log_{10}$ transformation of the ratio between the density of organisms present initially and after 5 days :

$$CGR = \log_{10} (N_5/N_0)$$

Data analysis

All raw data were entered into a Digital Systems MicroVax Model 3100 and converted to data sets using a SAS builder program (SAS Institute). The builder program was used to proof the raw data set. Statistical analyses were performed with SAS, and the degree of relation between variables was measured by the Pearson product-moment correlation coefficient (r).

RESULTS AND DISCUSSION

Responses to the questionnaire

A total of 79 utilities (45 %) responded to the questionnaires. Fifty one of the respondents indicated that they performed HPC analyses, typically with R2A at 28 °C, and those utilities were scheduled to participate in the survey. The participants represented 27 states from all regions of the United States and a Canadian Province *(fig. 1)*. Many utilities have multiple water sources which can be sampled separately,-and this enabled us to increase the number of different waters sampled in the survey to 79, including 26 groundwaters and 53 surface waters. In addition, 4 utilities were sampled with greater frequency, providing an estimate of temporal variability with 4 seasonal samples of the finished water, spatial variability with samples from 3 locations in the distribution system, and treatment effects, with samples from 3 points in the treatment process. Two ozone pilot plants were also included in the survey. In all, a total of 109 waters were tested.

Distributions of test parameters

Concentrations of DOC ranged from 203 to 4943 µg/L with a mean of 2069 and a median of 1988 *(fig. 2)*. The coefficient of variation for DOC analyses was typically between 1 to 3 %. The bimodal nature of the distribution resulted from a division between groundwater sources with a mean and median of 748 and 503 µg/L, respectively, and surface water sources with a mean and median of 2464 and 2278 µg/L, respectively. BDOC concentrations ranged from 1 to 1521 µg/L, with a mean and median of 289 and 242 µg/L, respectively *(fig. 2)*. Groundwaters sampled had mean and median BDOC concentrations of of 109 and 92 µg/L, respectively, meaning that 50 % of the concentrations were less than 100 µg/L. Surface water samples had mean and median BDOC concentrations of 336 and 296 µg/L, respectively, and less than 10 % were below 100 µg/L.

Concentrations of AOC calculated from yields on acetate-C (AOC_{ac}), ranged from 18 to 322, with a mean of 127 and a median of 114 µg/L *(fig. 2)*. The coefficient of variation for AOC analyses typically ranged from 5 to 20 %. Five of the finished waters had pH values between 9.3 and 10.4 which completely inhibited the growth of P-17 and NOX, and a sixth water had a pH of 8.4 which partially inhibited the growth of P-17. The data from these waters were excluded from the analysis. The groundwaters had mean and median AOC_{ac} concentrations of 71 and 52 µg/L, respectively, all values were below 200 µg/L, and 77 % were below 100 µg/L. In contrast, the surface waters had mean and median AOC_{ac} concentrations of 145 and 143 µg/L, respectively, and 62 % of the AOC_{ac} concentrations were above 100 µg/L.

Within the mixed cultures of P-17 and NOX, each species is able to oxidize compounds which the other cannot, but there is also competition for molecules which both can metabolize (VAN DER KOOIJ, 1990). It is unclear what factors, including treatment steps, influence the competitive outcome between P-17 and NOX in batch cultures of drinking water ; the exception is that ozonation is

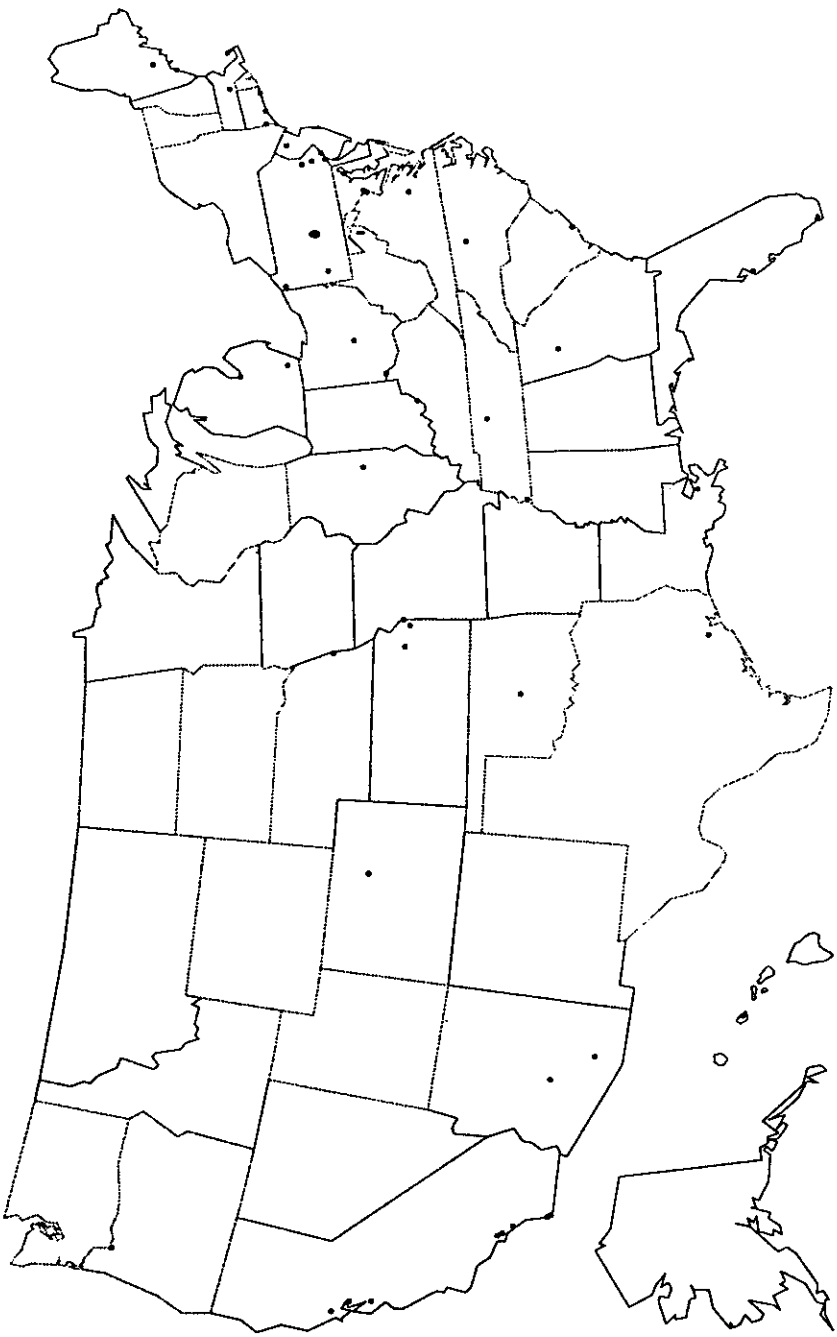

Figure 1 Map of the United States showing the location of water utilities included in the survey.
Situation géographique des réseaux de distribution inclus dans l'étude.

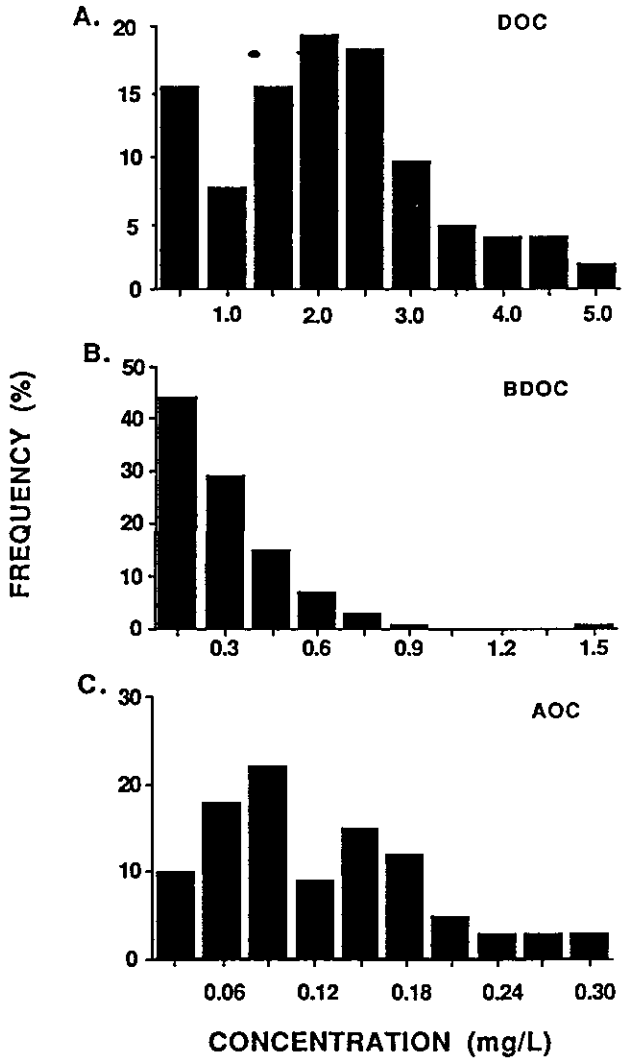

Figure 2 Frequency distribution of DOC, BDOC and AOC concentrations.
Distribution des fréquences de concentrations de COD, CODB et COA.

known to favor NOX. It seems reasonable to assume that the qualitative nature of the AOC is of primary importance. If we make that assumption and consider the relative densities of the two bioassay species underlying the AOC concentrations just presented, we find an indication of a qualitative as well as quantitative difference between the groundwater and the surface water AOC. Groundwater cultures were more likely to be dominated by P-17 than NOX. In groundwaters, the ratio of NOX/P-17 ranged from 0.08 to 5.19, with a mean and standard deviation of 1.8 ± 1.4. For the surface waters, that ratio ranged from 0.58 to 166, with a mean and standard deviation of 6.5 ± 21.

The influence of water source on DOC, AOC, and BDOC concentrations was also reflected in the CGR data, but to a much lesser extent. The great majority of all test waters (79 %) were not growth promoting, 7 % were strongly growth promoting (CGR > 1.0), all surface waters, and 14 % were moderately growth promoting (CGR = 0.5 – 0.99). The test waters with pH values between 9.3 and 10.4 also inhibited the *Enterobacter cloacae,* and these data were deleted from the analysis.

CGR incubations performed in 2 different vessels, 40-mL vials and 250-mL bottles, compared reasonably well *(fig. 3).* The mean for the vials (0.15) was higher than the mean for the bottles (0.08). Previous bioassay comparisons between incubation vessels also showed greater growth in smaller vessels with larger surface to volume ratios (KAPLAN and BOTT, 1989). This was interpreted as an example of the influence of surface area on bacterial growth, a phenomenon described by ZOBELL (1943) for *E. coli,* though questioned more recently (VAN LOOSDRECHT *et al.*, 1990).

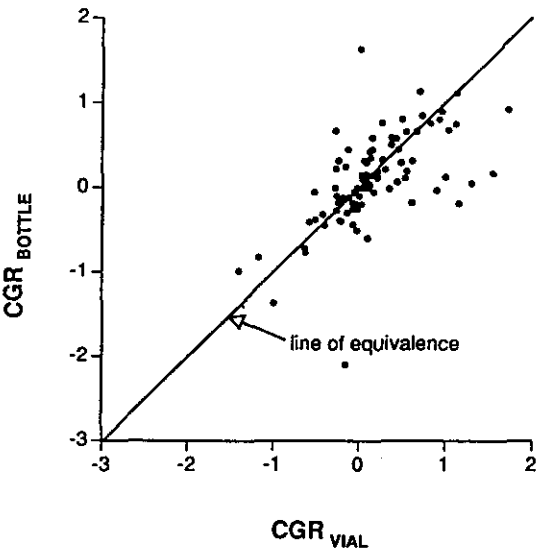

Figure 3 Influence of incubation vessel on CGR values.
Influence du flaconnage d'incubation sur la croissance bactérienne.

The coliform and HPC concentration data indicated very low numbers of organisms in the finished drinking waters. No coliforms were reported except for a single groundwater source with 25 coliforms/100mL, a DOC concentration of 190 µg/L, and an AOC concentration of 55 µg/L. No HPC organisms were detected in more than 50 % of the test waters, only three waters had greater than 100 cells/mL, and 29 waters had 5 or fewer cells/mL. These coliform and HPC data were reported for treatment plant effluents. It would probably have been more instructive to our understanding of the relationship between bacterial nutrients and regrowth if we had sampled for HPC at a point out in the distribution system.

Correlations between water quality parameters

AOC and BDOC showed significant correlations ($P<<0.01$) with DOC *(fig. 4)*. The correlation coefficient for AOC was $r=0.558$ and for BDOC, $r = 0.631$. Expressed as a percentage of DOC, BDOC ranged from 0.4 % to 52.8 %, and AOC ranged from 2.4 % to 44.0 %. The highest percentages were all limited to groundwaters, as AOC and BDOC concentrations in surface waters never exceeded 16 % and 39 %, respectively. The correlation of AOC and BDOC was also significant ($P<0.01$).

The AOC bioassay was developed by VAN DER KOOIJ *et al.* (1982) as an index of regrowth potential, not an absolute measure of organic carbon concentration. For convenience, the density of viable cells is converted to units of concentration expressed as acetate-C equivalents. Previous studies by VAN DER KOOIJ (1990) have established the yield of P-17 on a variety of compounds, and the yield of NOX on acetate and oxalate ; yield factors vary by as much as 4-fold, depending upon the substrate and the organism. Therefore the comparison of AOC and BDOC distributions in our survey, both expressed in the same concentration units of µg C/L, extends the AOC assay beyond its intended use, and implicitly assumes a yield factor, i.e. there is a presumption as to the qualitative nature of the AOC molecules. We have presented AOC concentrations using yield on acetate as the conversion factor (AOC_{ac}). When AOC_{ac} concentrations are compared to BDOC concentrations the value for BDOC nearly always exceed the AOC_{ac} concentrations *(fig. 5)*. This relationship may be explained as the difference between the range of catabolic capabilities contained in a bioassay using 2 species, NOX and P-17 and, and a bioassay using an unknown, but certainly greater number of species in the stream water inoculum.

However, some of the differences between AOC_{ac} and BDOC concentrations are probably related to the yield factor used for the AOC calculation. If the NOX contribution to the AOC concentration were calculated using the yield on oxalate, the resulting AOC_{ox} concentrations would increase and often exceed BDOC concentrations, particularly for the surface waters *(fig. 5)*. These 2 expressions of AOC concentration, AOC_{ac} and AOC_{ox}, can be considered as minimum and maximum estimates, resulting in a range of AOC concentrations which bracket the BDOC concentrations. Indeed, if we calculate AOC based on the oxalate yield, the correlation of AOC with BDOC improves to $r = 0.707$, but the important point to be understood is that some of the variation in the correlation of AOC and BDOC is related to the qualitative nature of the organic molecules present, not simply the catabolic ranges of the inocula.

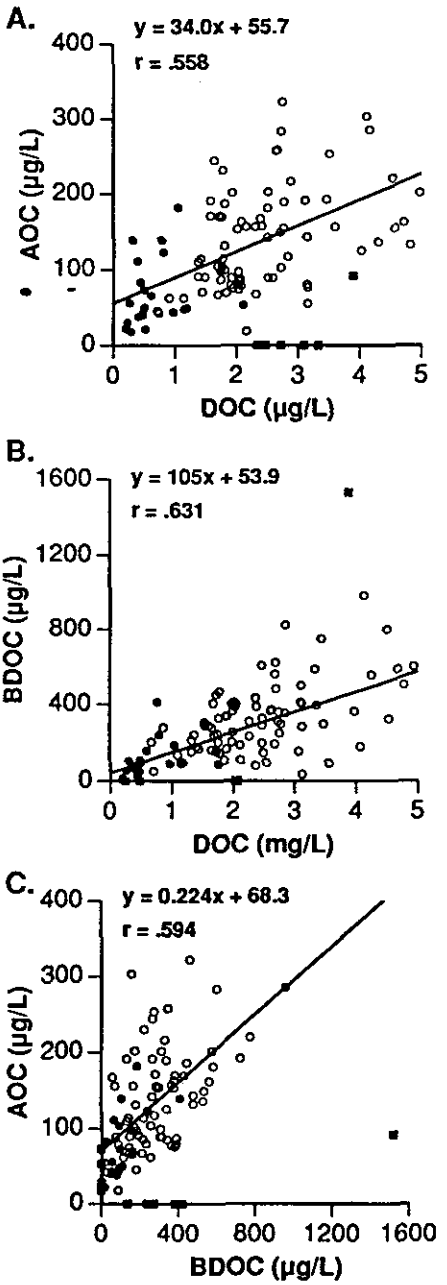

Figure 4 Correlations of : (A) AOC and DOC concentrations ; (B) BDOC and DOC concentrations ; and (C) AOC and BDOC concentrations. Groundwater (●), surface water (○), and data deleted from correlations (■).

Corrélations entre les concentrations de : (A) COA et COD ; (B) CODB et COD ; (C) COA et CODB. Eau souterraine (●), eau de surface (○) et valeurs éliminées pour le calcul de la corrélation (■).

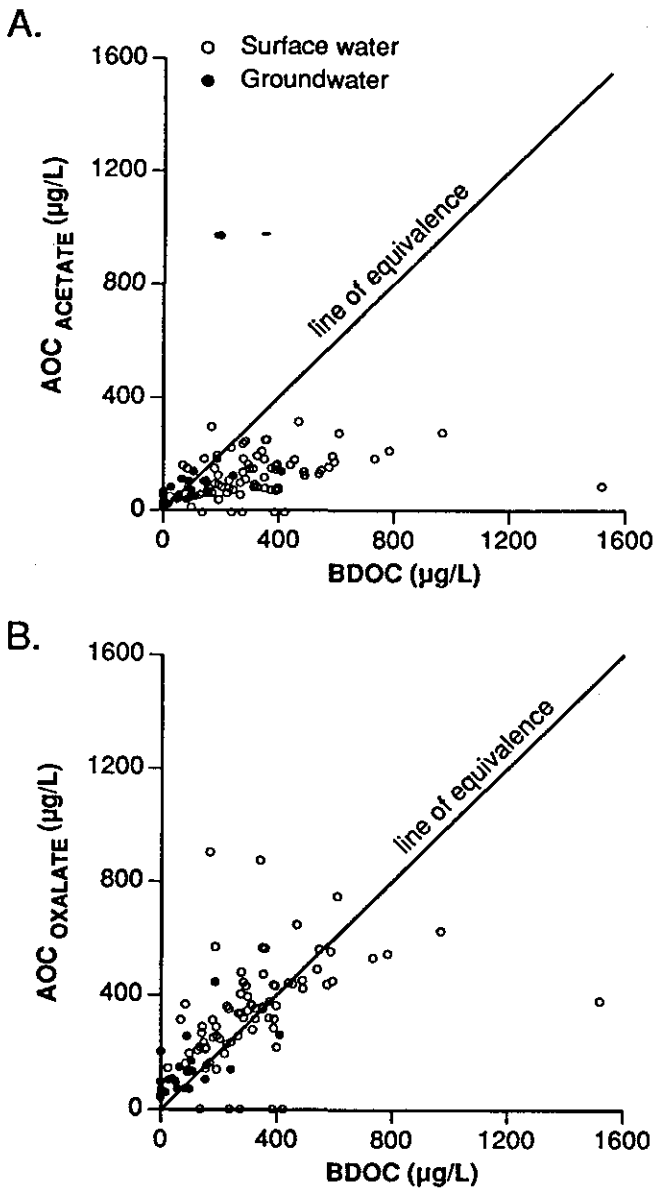

Figure 5 The influence of yield factors on AOC concentrations relative to BDOC *concentrations. Groundwater (●) and surface water (○).*

Influence des taux de conversion (y) sur la relation COA-CODB. Eau souterraine (●) et eau de surface (○).

Correlations between CGR and AOC or BDOC were poor, with values for the correlation coefficients not exceeding 0.15 *(fig. 6)*. This result differs from the earlier findings of RICE *et al.* (1991), who found a significant correlation between AOC concentrations and CGR with a correlation coefficient of 0.292. Some of the differences between that study and this survey include total numbers of test waters, AOC concentration in the test waters, and the AOC method. In the earlier study, 40 measures from fewer waters were made using only P-17 and 1-L flasks for incubation. RICE *et al.* (1991) reported a mean AOC concentration for treatment plant effluent waters of 127 µg/L, which is the same as the mean AOC concentration from our survey. However, we included NOX in the bioassay and used 40-mL vials. Both of these changes would have the effect of increasing the measured AOC concentrations we reported, suggesting that the waters sampled by RICE *et al.* (1991) were in fact higher in AOC concentration, on average, than the waters in our survey. If coliforms require higher concentrations of AOC for growth, this would explain some of the difference between our survey results and the results reported by RICE *et al.* (1991). Lastly, and possibly more importantly, the correlations found by RICE *et al.* (1991) were influenced in large part by the comparison of ozone exposed and unexposed water samples, something which was a minor factor in our survey.

Changes in AOC and BDOC concentrations during treatment

The application of AOC and BDOC measurements in the drinking water industry includes process optimization and process monitoring. When we looked at the patterns of DOC, AOC, and BDOC concentration changes during treatment at 4 different utilties, DOC decreased in all cases *(table 1)*. Both AOC and BDOC decreased through treatment only at utility 177. Concentrations of AOC decreased at utility 125 and BDOC declined at utilities 47 and 138. We suspect that some of the disparities in AOC and BDOC patterns may be related to chemical or biological interferences in the application of these bioassays to surface water prior to full treatment. Another problem in trying to relate patterns of AOC and BDOC changes involves the question of yield. As previously discussed, the factor chosen for calculation of AOC concentrations will change the AOC concentration, and the ratio of bioassay organisms at different points in the treatment often changed dramatically. For example, in utility 047, the ratio of NOX to P-17 densities measured in raw, filtered, and finished water changed from 3.3 to 48.3 to 3.8, respectively. The shift from P-17 to NOX in the AOC bioassay following ozonation is well known, but in the surface waters under consideration here, ozonation was not a factor. As the distribution of bioassay organisms changes, the pattern of AOC concentrations would also change if, for example AOC_{ox} rather than AOC_{ac} were calculated.

Temporal and seasonal patterns of DOC, AOC, and BDOC concentrations

When we considered only the treatment plant effluent, such as in the survey of temporal changes related to seasons or spatial changes related to transit through a distribution system *(table 1)*, there were no consistent patterns of

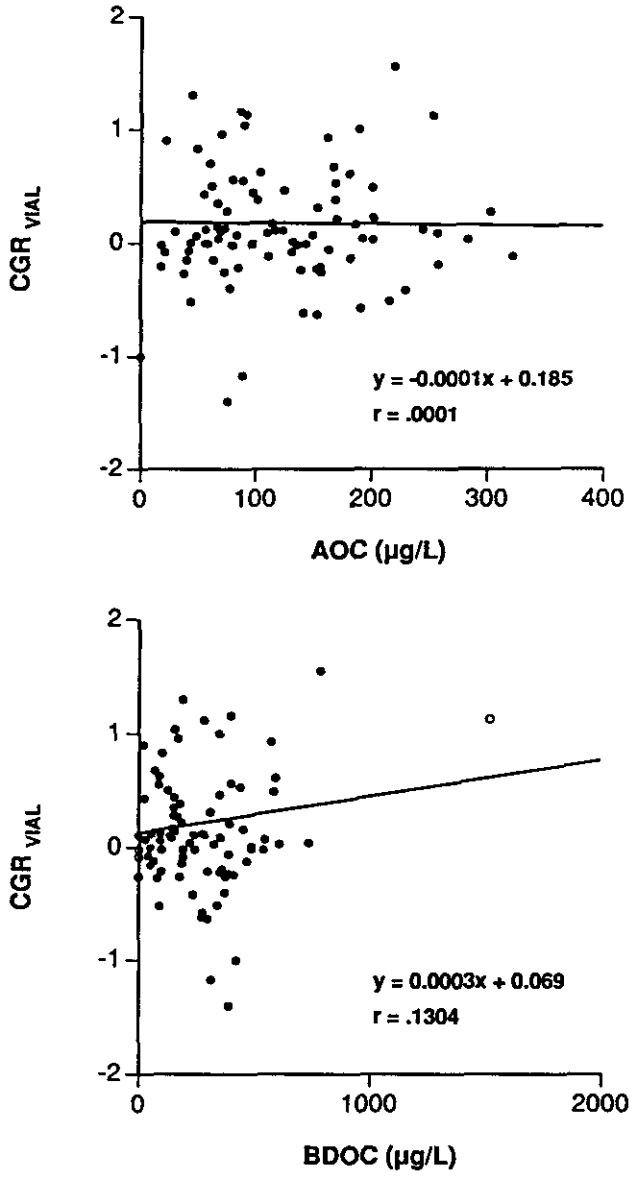

Figure 6 Correlations of : (A) CGR and AOC concentrations ; and (B) CGR and BDOC concentrations. Open symbols are data excluded from the correlations.

Corrélations entre : (A) croissance bactérienne et concentrations de COA ; (B) croissance bactérienne et concentrations de CODB. Les symboles ouverts correspondent aux données pour le calcul de la corrélation.

Table 1 Changes in DOC, BDOC and AOC concentrations at 4 utilities resulting from treatment, season, or transport in the distribution system.

Tableau 1 *Variations des concentrations de COD, CODB et COA, résultant du traitement, de la saison ou du transport dans le réseau, pour 4 sociétés de distribution.*

Variable	UTILITY CODE											
	047			177			125			138		
	DOC	BDOC	AOC	DOC	BDOC	AOC	DOC	BDOC	AOC	DOC	BDOC	AOC
Treatment **												
Raw water	3124	25	55	3481	285	253	3087	143	191	3122	392	76
Filter effluent	2132	98	18	3748	350	189	2689	180	102	2710	469	322
Plant effluent	2000	0	73	2364	68	167	2390	375	157	2640	360	258
Season												
Summer	2055	389	163	1905	325	201	2616	354	257	3890	1521	92
Autumn	2000	0	73	2364	68	167	2390	375	157	2640	360	258
Winter	1741	148	114	1450	157	90	1334	140	110	1765	191	131
Spring	1865	97	79	1701	227	86	1782	456	186	1998	352	85
Location												
Treatment plant	1865	97	79	1701	227	86	1782	456	186	1998	352	85
Distribution 1	1992	192	97	1775	317	99	1684	395	170	2029	399	87
Distribution 2	1997	242	80	1336	203	91	1730	441	169	2039	373	78

* Concentrations of DOC, BDOC and AOC are in units of µg C/L.

** Treatments for each utility are listed sequentially as follows :

047 – alum/polymer coagulation ; anthracite/sand filtration ; chorination, fluoride ; zinc metaphosphate ; sodium hydroxide.

125 – alum/polymer coagulation ; chlorination, lime, fluoride ; chlorination ; rapid sand filtration ; chlorination.

138 – chlorination, alum, lime ; chlorination ; anthracite/sand filtration ; ammonia ; fluoride.

177 – potassium permanganate, powdered activated carbon, sulfur dioxide, lime ; copper sulfate ; chlorination ; alum/settling ; anthracite/sand filtration ; chloramine ; zinc and sodium phosphate ; ammonia.

change. No dramatic reductions occurred during transport through the distribution systems as would be expected if biological stabilization were occurring. In fact, with each of these surface water supplies, the variation between seasonal values exceeded the range of concentrations found during distribution. Changes in the ratio of bioassay organisms did occur seasonally or between points in a distribution system, but these changes were typically not dramatic.

CONCLUSION

The data which we have collected in this survey represent the first comparison of AOC and BDOC methods applied to a wide range of water types in North America. We have found that the source of water, more so than treatments of the waters included in this survey, had a strong influence on AOC and BDOC concentrations. This is related to the quantitative and qualitative differences between groundwaters and surface waters, and the fact that most utilities in North America do not optimize their treatment for the removal of AOC or BDOC. Utilities do optimize treatment to eliminate organisms in the finished water, and their success in accomplishing that goal is demonstrated by the coliform and HPC data for finished waters.

It has been suggested that the AOC assay based upon biomass measurements and the BDOC assay based upon DOC measurements may target different objectives in the treatment process (HUCK, 1990). The former addresses concerns for bacterial regrowth while the latter addresses reduction in chlorine demand or disinfection by-product formation. We agree that the BDOC assay provides information about both the labile and refractory components of DOC. However, we would argue that our data also indicate that if water utilities should choose to try and reduce the concentrations of nutrients for bacterial growth through treatment, that the AOC and BDOC methods are complementary techniques that can be applied to the problem.

The AOC technique as developed by VAN DER KOOIJ *et al.* (1982) is extremely sensitive. Modifications of the procedure such as vessel type (KAPLAN and BOTT, 1990), inoculum density (KAPLAN and BOTT, 1989), or test parameter, such as ATP (JAGO and STANFIELD, 1989) or direct microscopy (BRADFORD *et al.*, 1991) have been proposed to simplify and shorten the assay, but it is doubtful that the method can or needs to be made more sensitive. It is possible, however, that with a better understanding of the competitive interactions of the bioassay organisms, that additional qualitative information can be gleaned from the AOC assay. The BDOC technique as developed by HASCOET *et al.* (1986) and modified by JORET and LEVI (1986) proports to have a detection limit of approximately 100 to 200 µg C/L. We believe that with improved techniques of organic carbon analysis, used here, that the detection limit is < 50 µg C/L. With possible modifications of the assay to utilize fixed biofilm reactors (RIBAS *et al.*, 1991), the detection limit and duration of the BDOC assay can probably be reduced further.

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